REPORT DOCUMENTATION PAGE

Form Approved OMB NO. 0704-0188

1. REPORT DATE (DD-MM-YYYY)	2. REPORT TYPE	3. DATES COVERED (From - To)
	New Reprint	-

4. TITLE AND SUBTITLE
Remote detection of plant physiological responsesto TNT soil contamination

5a. CONTRACT NUMBER
W911NF-06-1-0074

5b. GRANT NUMBER

5c. PROGRAM ELEMENT NUMBER
611102

6. AUTHORS
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5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

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Richmond, VA 23298 -0568

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES)
U.S. Army Research Office
P.O. Box 12211
Research Triangle Park, NC 27709-2211

10. SPONSOR/MONITOR'S ACRONYM(S)
ARO

11. SPONSOR/MONITOR'S REPORT NUMBER(S)
49364-EV.6

12. DISTRIBUTION AVAILIBILITY STATEMENT
Approved for public release; distribution is unlimited.

13. SUPPLEMENTARY NOTES
The views, opinions and/or findings contained in this report are those of the author(s) and should not contrued as an official Department of the Army position, policy or decision, unless so designated by other documentation.

14. ABSTRACT
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15. SUBJECT TERMS
Hyperspectral reflectance . Chlorophyll fluorescence . Photosynthesis

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	15. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT	b. ABSTRACT	c. THIS PAGE			Donald Young
UU	UU	UU	UU		19b. TELEPHONE NUMBER: 804-828-1562

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REPORT DOCUMENTATION PAGE (SF298)
(Continuation Sheet)

Continuation for Block 13

ARO Report Number 49364.6-EV
Remote detection of plant physiological respons ...

Block 13: Supplementary Note

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Plant Soil (2010) 329:239–248
DOI 10.1007/s11104-009-0148-1

REGULAR ARTICLE

Remote detection of plant physiological responses to TNT soil contamination

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Received: 19 June 2009 / Accepted: 17 August 2009 / Published online: 29 August 2009

Abstract Our study was aimed at understanding physiological responses to trinitrotoluene (TNT) soil contamination, and using optical methods to detect TNT-induced stress in a woody plant prior to visible changes. *Myrica cerifera* plants were potted in soil concentrations of TNT ranging from 30–500 mg kg^{-1}. Physiological measurements were significantly affected by TNT exposure at all treatment levels, and photosynthetic decline likely resulted from metabolic impairment rather than stomatal closure as the experiment progressed. Several reflectance indices were able to detect TNT-induced stress before any changes in chlorophyll concentrations occurred. The most sensitive index was the simple ratio R_{761}/R_{757} which is linked to fluorescence *in-filling* of the 0_2 atmospheric absorption. Changes at R_{740}/R_{850} and R_{735}/R_{850} may be attributed to both fluorescence and structural characteristics of leaf anatomy in the near infrared region. This could have been influenced by transformation and conjugation of TNT metabolites with other compounds. chlorophyll index (CHL) or in the water band index (WBI_{970}), which are indices typically associated with drought stress, and may provide a means of separating stress due to explosives. Further studies need to be conducted with a combination of stressors (TNT and natural) to determine if responses are in fact generalized or if any of these changes are separable from natural stress.

Keywords Trinitrotoluene · Hyperspectral reflectance · Chlorophyll fluorescence · Photosynthesis

Abbreviations

TNT	Trinitrotoluene
PPFD	photosynthetic photon flux density
g_{wv}	stomatal conductance
A_{Net}	net photosynthetic rate
F_o	minimal fluorescence in dark-adapted leaves
F_m	maximal fluorescence in dark-adapted leaves
F_v/F_m	maximum quantum use efficiency of PSII in the dark-adapted state
F'_o	minimal fluorescence in light-adapted leaves
F'_m	maximal fluorescence in light-adapted leaves
F_s	steady-state fluorescence
$\Delta F/F'_{m,}$	fraction of absorbed photons that are used for photochemistry in a light-adapted leaf
F'_v/F'_m	effective quantum use efficiency of PSII in the light-adapted state
PSII	photosystem II
WBI	water band index

Responsible Editor: Liz Shaw.

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CHL chlorophyll index
PRI physiological reflectance index

Introduction

The concept of using vegetation as sentinels to indicate presence or absence of toxic contaminants is not new and could potentially provide an ideal mechanism for large-scale detection. Vegetation indices have been developed based on variations in plant reflectance, which sense changes in biomass, chlorophyll content and leaf water status; however, differences in many of these indices often take place after visible signs of stress have occurred. It has also been difficult to positively attribute spectral phenomena to a specific plant-contaminant interaction (Carter and Knapp 2001); and until recently there was no link between spectral indices and photosynthetic function.

Advances in fluorescence spectroscopy and reflectance-derived fluorescence have made possible earlier detection of stress in plants (Zarco-Tejada et al. 2003, 2009; Evain et al. 2004), especially before changes in chlorophyll content are visible. This research approach has been applied to many areas of environmental stress, most notably drought, salinity, nitrogen concentrations and other nutrient deficiencies (Morales et al. 2000; Dobrowski et al. 2005; Campbell et al. 2008; Naumann et al. 2008a, 2009). Despite these advances in remote sensing technology, understanding and application to anthropogenic stressors, particularly explosives, remains limited. As our understanding of plant responses to various contaminants improves, it may be feasible to use naturally occurring vegetation to monitor the environment, particularly in regard to explosive compounds, which could have tremendous military and environmental impacts.

Research in the field of TNT detection and remediation has been driven by the need to clean up contaminated sites due to munitions production and processing facilities as well as military activities (Best et al. 2008). Many studies have focused on seedling germination and early growth in TNT contaminated soils (Gong et al. 1999; Robidoux et al. 2003; Ali et al. 2006), yet few studies investigate the effect of TNT on mature plants. Although it is well documented that reductions in biomass are a result of TNT exposure (Krishnan et al. 2000; Robidoux et al. 2003), research examining the physiological responses of these plants are limited, particularly to studies of plants grown in culture or hydroponic solutions, which do not translate as readily to field application. By investigating the effect of TNT on a variety of species at different phenological stages, we may be able to find methods for exploiting plants as sentinels to detect stress caused by landmines that are likely leaching chemicals into the soil.

While numerous studies have looked at the uptake and biotransformation of TNT in plants (Medina and McCutcheon 1996; Sens et al. 1999; Best et al. 2008), little is known about how these compounds and associated metabolites directly impact the photosystem, resulting in decreased biomass and chlorosis. It has been proposed that xenobiotics are taken up and carried to the leaves through transpiration, transformed and conjugated with other compounds and compartmentalized in the vacuole, cell wall or lignin (Trapp and McFarlane 1995). Reflectance changes in both the cell wall structure and photosynthetic functioning of plants exposed to TNT could be used for potential remote detection of contaminated soils.

Research involving TNT effects on plants is largely focused on herbaceous species or woody plants grown in hydroponic media, while examination of effects on woody plants growing in soil is lacking. There is a need to understand how other growth forms respond to TNT contamination for ease of scaling up from laboratory studies to landscape level applications. We used woody shrub saplings for our investigation to aid in future field studies where mature plants may be growing on TNT contaminated soils. The objectives of our study were to (1) understand the physiological responses of a woody shrub grown in soil contaminated with various concentrations of TNT and (2) to use hyperspectral remote sensing and chlorophyll fluorescence to possibly detect uptake in plant leaves in a laboratory setting. Investigating the use of remote sensing methods to identify TNT contamination in laboratory studies may facilitate detection in larger scale field studies.

Materials and methods

Soil contamination

For TNT treatments, 200 ml acetone containing different concentrations of TNT was added and

homogenized with 4.2 kg of a low nitrogen soil to obtain initial concentrations of 30 mg, 100 mg, 250 mg and 500 mg of TNT kg^{-1} dry soil. For control plants we used soil treated only with 200 ml of acetone. Control and TNT treated soils samples were kept 72 h in the dark to avoid photodegradation of TNT and to evaporate the acetone from the soil (Ali et al. 2006).

Plant materials

Myrica cerifera L., Myricaceae, (wax myrtle) was chosen as the study species because the physiology and natural stress response has been well quantified. *Myrica cerifera* is an evergreen shrub that forms extensive, dense thickets and is the dominant woody species along the Atlantic Coast (Ehrenfeld 1990; Young et al. 1994). Coastal military facilities are frequently inhabited with *M. cerifera*, and this could provide future field opportunities in contaminated soils. Fruits of *M. cerifera* were collected from Hog Island (37° 40′N; 75° 40′W), a barrier island located on the Eastern Shore of Virginia, and crushed with a mortar and pestle to break the waxy coating and scarify seeds. Seeds were sown in transparent plastic trays filled with one inch of Perlite growth medium, and watered as necessary with water. Plants with at least three sets of secondary leaves were transplanted into 2 L plastic pots and grown for at least 5 months prior to experimentation. Plants were grown in a Conviron environmental chamber (CMP 3244, Controlled Environments Limited, Asheville, NC) under a photosynthetic photon flux density (PPFD) of approximately 700 μmol m^{-2} s^{-1}, 48% relative humidity, a photoperiod of 14 h, and a day/night temperature of 30/25°C. Saplings were 50 cm at the beginning of the experiment and kept continually moist, but well-drained throughout the experiment.

Measurements of gas exchange and fluorescence

Plant responses to TNT treatments were quantified by measuring stomatal conductance to water vapor (g_{wv}), leaf net photosynthesis (A_{Net}), leaf fluorescence and leaf reflectance (n=5 per treatment). Measurements were conducted mid-day (1,000–1,400 h) once a week for 9 weeks. Rates of stomatal conductance and leaf net photosynthesis were measured using a portable infrared gas analyzer at a photosynthetic photon flux density of 700 μmol m^{-2} s^{-1}, 48% relative humidity, and 28°C air temperature (LI- 6400, LI-COR Biosciences, Inc., Lincoln, NE).

Light-adapted and dark-adapted measurements of chlorophyll fluorescence were conducted on the fourth or fifth fully expanded leaf of each plant using a pulse amplitude modulated leaf chamber fluorometer (LI- 6400, LI-COR Biosciences, Inc., Lincoln, NE). Minimal fluorescence values in the dark-adapted state (F_o) were obtained by application of a low intensity far-red measuring light source (740 nm), while maximal fluorescence values (F_m) were measured after applying a saturating light pulse of 8,000 μmol m^{-2} s^{-1} (630 nm). Minimum (F'_o) and maximum (F'_m) values of fluorescence in the light-adapted state at 700 μmol m^{-2} s^{-1} were also obtained in this manner. Using these parameters, the following ratios were calculated: $\Delta F/ F'_m=(F'_m-F_s)/ F'_m$, quantum yield of xanthophyll-regulated thermal energy dissipation and maximum quantum use efficiency of photosystem II (PSII) in the dark-adapted state, $F_v/F_m = (F_m-F_o)/F_m$. Leaves were dark-adapted for 30 min using dark-adapting leaf clips (LI-COR Biosciences, Inc., Lincoln, NE) for F_v/F_m measurements.

Chlorophyll concentrations

Leaf samples were collected by punching forty 0.32 cm^2 disks from each plant at the end of the experiment. Chlorophyll concentrations were determined based on methods recommended by Šesták (1971) by extracting chlorophyll using a 100% acetone solution. Samples were then ground with a mortar and pestle, filtered, and analyzed using a Spectronic 21 spectrophotometer. Chlorophyll concentrations were calculated using equations described by Holm (1954).

Reflectance measurements

Measurements of leaf reflectance were taken concurrently with physiological and fluorescence measurements. An ASD FieldSpec Pro reflectance radiometer (Analytical Spectral Devices, Inc., Boulder, CO) was used to measure the spectral reflectance of leaves between 350 nm–2,500 nm. The ASD spectral resolution is approximately 1–3 nm from the visible to the short-wave infrared. Laboratory leaf measurements were collected perpendicular to the fore-optic using a 3,200°K lamp as an illumination source. The fore-optic of the radiometer was held nadir at a nominal distance of

0.25 m using an 8° field-of-view. To acquire a representative value, multiple spectra were collected and averaged for each leaf. Data were reduced from binary using the manufacturer's software. A NIST spectralon reflectance standard was used as a white reference to optimize instrument gains prior to each canopy measurement. This standard provides a near 100% lambertian reflectance surface for calibration. Using the resulting reflectance values, several reflectance indices examining potential changes in pigments, reflectance-derived fluorescence and water content were calculated (Table 1).

Statistical analysis

Variations in photosynthetic characteristics, stomatal conductance, fluorescence, reflectance indices and chlorophyll concentrations among TNT treated plants relative to control plants were analyzed with one-way analysis of variance for each stress experiment (Zar 1999). Dunnett's multiple comparisons (α=0.05) identified significant differences in treatment plants relative to controls.

Results

Gas exchange and fluorescence measurements

Physiological stress in *M. cerifera* was induced by TNT as seen in stomatal conductance, photosynthesis and fluorescence measurements. A decrease in stomatal conductance was seen in plants treated with 100 mg, 250 mg and 500 mg TNT kg^{-1} by week 2 (F=9.79, P< 0.0001; Fig. 1; Table 2). By week 3, all treatments had significantly lowered stomatal opening relative to control plants (F=6.77, P=0.0013); however, stomatal conductance values did not remain low over the course of the experiment for plants treated with 30 mg TNT kg^{-1}. Values remained lower than controls throughout the course of the experiment for all other treatments, with the exception of week 4, where no significant differences were observed (F=1.90, P=0.1501). Measurements of photosynthesis revealed significant reductions in all treatments by week 1 (F=12.68, P<0.0001) and remained lower than controls throughout the experiment (Fig. 1; Table 2), despite weeks where stomatal conductance values did not differ from controls. Stomatal control did not appear to be the main mechanism for lowering photosynthesis in TNT treated plants (F=81.78, P<0.0001, r^2=0.33; Fig. 2).

Both light- and dark-adapted measurements were quantified to assess stress due to TNT uptake. An initial reduction in $\Delta F/F'_m$ occurred in plants treated with 250 mg kg^{-1} and 500 mg kg^{-1} by week 2 (F=7.50, P=0.0007; Fig. 3; Table 2) and remained through week 3. Recovery occurred for both treatments by week 4 (F=2.84, P=0.0517). By week 6, plants treated with 100 mg kg^{-1}, 250 mg kg^{-1} and 500 mg kg^{-1} had significantly lower $\Delta F/F'_m$ than controls (F=5.77, P=0.0030) and remained lower throughout the experiment. Plants treated with 30 mg TNT kg^{-1} exhibited lower $\Delta F/F'_m$ values during week 7 only (F=19.68, P <0.0001). Dark-adapted fluorescence values (F_v/F_m) decreased in plants treated with 250 mg and 500 mg TNT kg^{-1} dry soil by week 5 (F=13.02, P<0.0001). Plants at 250 mg kg^{-1} recovered by week 6, however, F_v/F_m remained significantly lower than controls for the remainder of the experiment in

Table 1 Selected vegetation indices used in statistical analyses

Reflectance index	Formula	Reference
Water band index (WBI_{970})	R_{970}/R_{900}	(Peñuelas et al. 1993)
Chlorophyll index (CHL)	$(R_{750}-R_{705})/(R_{750}+R_{705})$	(Gitelson and Merzlyak 1996)
Structural insensitive pigment Index (SIPI)	$(R_{800}-R_{445})/(R_{800}+R_{680})$	(Peñuelas et al. 1995)
Physiological reflectance Index (PRI)	$(R_{531}-R_{570})/(R_{531}+R_{570})$	(Gamon et al. 1992)
Green normalized difference Vegetation index (Green NDVI)	$(R_{801}-R_{550})/(R_{801}+R_{550})$	(Gitelson et al. 1996)
Curvature index (CI)	$R_{683}^2/(R_{675}*R_{691})$	(Zarco-Tejada et al. 2000)

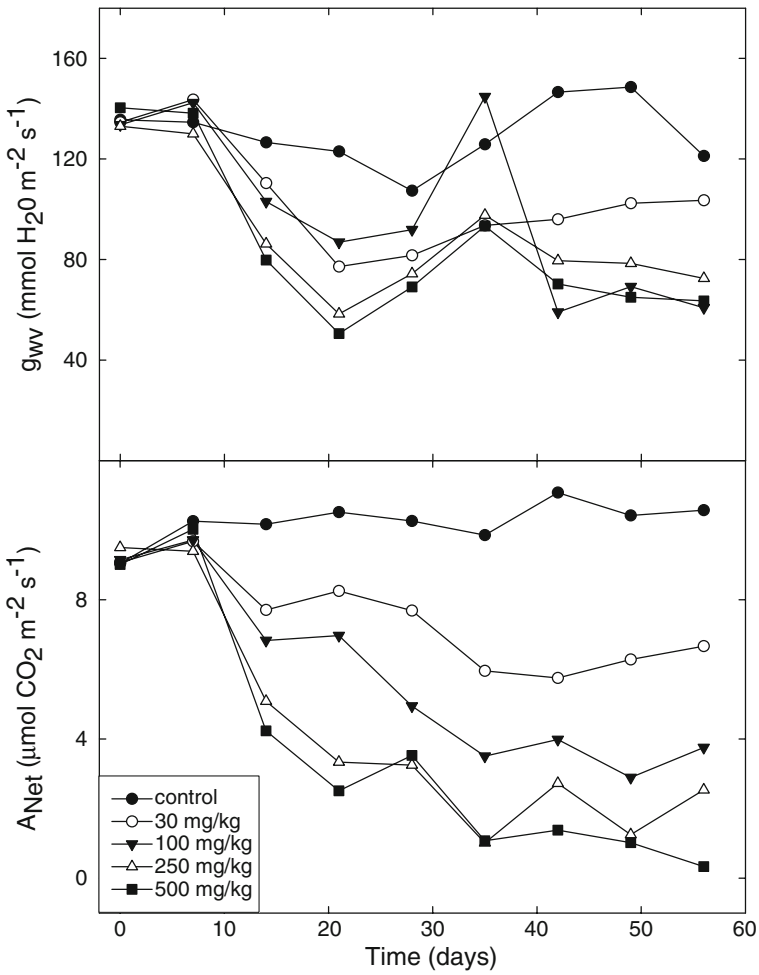

Fig. 1 Effects of TNT contaminated soil on stomatal conductance (g_{wv}) and net photosynthesis (A_{Net}) for *Myrica cerifera*. Symbols represent means for control and treatment plants

plants treated at 500 mg kg^{-1} (Fig. 3; Table 2). These significant differences occurred before any visible signs of stress.

Chlorophyll content

Chlorophyll *a* concentrations decreased with increasing stress, ranging from 316±16 to 270±8 mg m^{-2}, but there were no significant differences among treatments (F=2.26, P=0.100; Table 3). Chlorophyll *b* concentrations ranged from 193±41 to 244±16 mg m^{-2} without significant differences among treatments (F=1.72, P=0.186). There were no significant differences among TNT treatments for total chlorophyll (F=2.12, P=0.118) or the chlorophyll *a:b* ratio (F= 0.84, P=0.516; Table 3).

Reflectance

TNT induced stress was apparent in multiple indices during weeks 6, 7 and 8 (Table 4). The water band index (WBI) and chlorophyll index (CHL) did not change throughout the experiment. The physiological reflectance index (PRI), which is linked to photosynthetic functioning, was successful at detecting stress in plants at 500 mg kg^{-1} from week 6 and through the remainder of the study (Table 4). By the end of the experiment, PRI was significantly lower in plants at 250 mg kg^{-1} and 500 mg kg^{-1}. Several indices that are considered to be reflectance-derived fluorescence showed significant changes in the 500 mg kg^{-1} treatment. Most notable is the simple ratio R_{761}/R_{757}, which remained significantly lower

Table 2 Summary of *P*-values from Dunnett's multiple comparisons, which identified significant differences in treatment plants relative to controls. Bold numbers indicate significance at α=0.05

Week	Treatment level (mg kg^{-1})	Stomatal conductance	Net photosynthesis	$\Delta F/F'_m$	F_v/F_m
1	30	0.99	**0.02**	0.87	1.00
	100	1.00	**0.02**	0.97	**0.02**
	250	0.88	**0.01**	1.00	0.74
	500	1.00	**0.04**	0.94	**0.00**
2	30	0.21	**0.04**	0.99	0.98
	100	**0.04**	**0.01**	0.98	0.92
	250	**0.00**	**0.00**	**0.03**	0.61
	500	**0.00**	**0.00**	**0.00**	1.00
3	30	**0.03**	**0.01**	0.99	0.99
	100	0.09	**0.00**	0.99	0.09
	250	**0.00**	**0.00**	**0.02**	0.10
	500	**0.00**	**0.00**	**0.00**	0.73
4	30	0.31	**0.03**	0.07	0.98
	100	0.72	**0.01**	0.90	0.41
	250	0.14	**0.00**	0.99	0.08
	500	0.07	**0.00**	0.97	0.26
5	30	0.29	**0.00**	0.07	0.99
	100	**0.03**	**0.00**	0.18	0.98
	250	**0.04**	**0.00**	**0.00**	**0.00**
	500	**0.03**	**0.00**	0.51	**0.00**
6	30	0.11	**0.00**	0.95	0.53
	100	**0.00**	**0.00**	**0.01**	0.98
	250	**0.02**	**0.00**	**0.03**	0.16
	500	**0.01**	**0.00**	**0.01**	**0.00**
7	30	**0.00**	**0.00**	**0.03**	0.98
	100	**0.00**	**0.00**	**0.00**	0.97
	250	**0.00**	**0.00**	**0.00**	1.00
	500	**0.00**	**0.00**	**0.00**	**0.00**
8	30	0.37	**0.00**	0.98	0.98
	100	**0.00**	**0.00**	**0.00**	0.09
	250	**0.00**	**0.00**	0.06	0.99
	500	**0.00**	**0.00**	**0.00**	**0.00**

in multiple treatments from weeks 6–8, and was significantly lower in all treatment levels during week 7 (Table 4).

Discussion

TNT affects photosynthesis and the light reactions, which has been documented as changes in biomass and degradation of chlorophyll in leaves (Thompson et al. 1998; Best et al. 2008). It is well known that plants take up TNT and convert it to various metabolites in the leaves, stems and roots (Sens et al. 1999). Very few studies have examined the effect of TNT on physiological responses of plants (Thompson et al. 1998; Ali et al. 2006), thus the exact mechanism with which TNT causes perturbations in the photosystem remains unknown. By furthering our understanding of how TNT affects plants, we may be able to develop methods for detecting explosives uptake in plants which would have enormous benefits in both military and environmental applications. Our study was aimed at understanding both physiological responses to TNT contamination, as well as using optical methods to detect

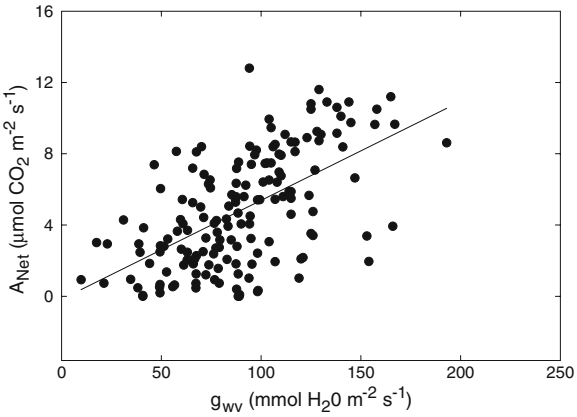

Fig. 2 Relationship between stomatal conductance (g_{wv}) and net photosynthesis (A_{Net}) for *Myrica cerifera* under TNT treatment

TNT-induced stress in a woody plant prior to visible changes.

Response and growth of plant in TNT contaminated soils appears to be species dependent (Scheidemann et al. 1998). Our results indicate that *M. cerifera* was able to grow in concentrations up to 500 mg kg^{-1} with no obvious signs of visible damage to leaves. Other studies have reported growth of plants in soils up to 750 mg kg^{-1} with no obvious effects (Mueller et al. 1995). Reductions in biomass, although not measured, were apparent in plants treated with higher TNT concentrations relative to controls by the end of the experiment. Despite low photosynthetic rates, plants at all concentrations continued to grow.

Partial stomatal closure was observed in response to TNT exposure in all treatments, which may decrease the influx of CO_2 to maintain photosynthesis. After a few weeks of exposure, some plants increased

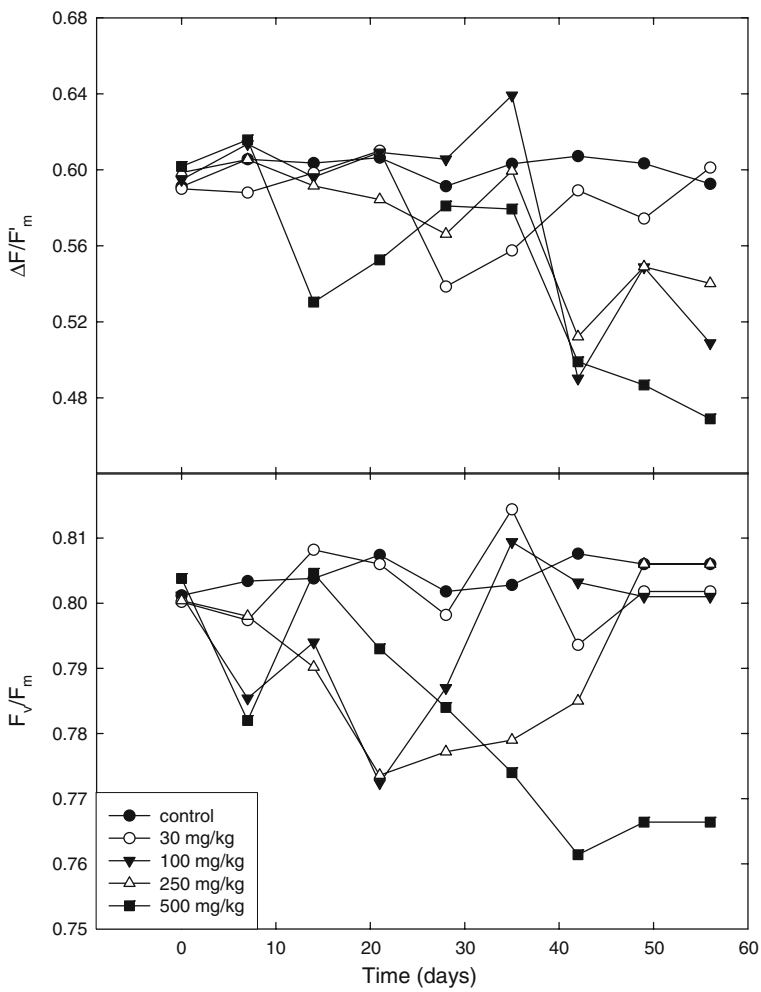

Fig. 3 Changes in the fluorescence measurements $\Delta F/F'_m$, and F_v/F_m for *Myrica cerifera* under TNT treatments over time. *Symbols* represent means for control and treatment plants

Table 3 One-way analysis of variance of chlorophyll concentrations among TNT treatments. Values are means ± 1 SE

	TNT concentration (mg kg^{-1} soil)				
	0	30	100	250	500
Chl *a* (mg m^{-2})	306±11	304±7	301±22	273±12	270±4
Chl *b* (mg m^{-2})	237±9	231±6	248±31	193±18	210±6
Total chlorophyll (mg m^{-2})	543±20	536±14	550±54	466±22	480±10
Chlorophyll *a*:*b* (mg m^{-2})	1.3±0.0	1.3±0.0	1.2±0.1	1.5±0.2	1.3±0.0

stomatal opening before closing again. Despite these fluctuations in stomatal opening, photosynthesis continued to decline. This is a marked difference from physiological responses to drought, salinity, flooding and herbicide in *M. cerifera* where reduced photosynthesis is tightly coupled to stomatal closure (Naumann et al. 2007, 2008b). In this study, there was not a strong link between stomatal conductance and photosynthesis, especially as the experiment progressed, indicating that metabolic processes were affected by TNT and thus limiting the photosystem. This could be a distinguishing feature of TNT induced physiological stress from natural stresses.

Experiments with green algae have shown a genetic response that affects the electron transport chain and which can result in oxidative stress (Patel et al. 2004). However, oxidative stress is a response observed in plants due to various types of stresses, both biotic and abiotic. A recent study has indicated a deterioration of the water splitting system of PSII, as determined by fluorescence kinetics, and was distinguishable from herbicide induced effects (Ali et al. 2006). This suggests that further investigation into the fluorescence signal may facilitate the detection of specific stressors in laboratory studies.

Another notable difference in response to TNT from natural stresses was seen in the fluorescence response. F_v/F_m decreased significantly in plants at higher concentrations before any visible damage was seen in the leaves. The reduction in F_v/F_m at 500 mg TNT kg^{-1} indicates that these plants were photoinhibited. For *M. cerifera*, this is the first stress that has caused a response in F_v/F_m before visible effects (Naumann et al. 2007, 2008b) and could potentially be a discriminating factor for separating natural from anthropogenic stress. Despite changes in fluorescence, there were no significant changes in chlorophyll content among any of the treatments.

In comparison, measurements of hyperspectral reflectance were able to distinguish stress at multiple concentrations of TNT exposure from control plants. PRI and R_{740}/R_{850} were able to detect stress at week 6 and throughout the remainder of the experiment. PRI is linked to changes in xanthophyll cycle pigments as a mechanism of non-radiative energy dissipation (Gamon et al. 1992; Demmig-Adams and Adams 1996) and has been successfully applied in various

Table 4 Summary of ANOVA of reflectance indices. F, *P* values are reported for significant differences among treatments. Bold *P* values indicate significance at 500 mg TNT kg^{-1} soil compared to control plants. Other differences are denoted with letters

Reflectance index	Week 6	Week 7	Week 8
WBI	2.34, 0.09	0.62, 0.66	0.88, 0.49
CHL	2.70, 0.06	0.53, 0.72	2.38, 0.10
SIPI	2.92, **0.04**	0.29, 0.31	0.12, 0.97
PRI	3.61, **0.02**	2.90, **0.04**	2.94, **0.04**[b]
GreenNDVI	3.39, **0.03**	0.55, 0.69	2.66, 0.06
R_{685}/R_{655}	3.17, **0.03**	1.65, 0.20	1.86, 0.16
R_{680}/R_{630}	3.40, **0.04**	2.22, 0.17	2.29, 0.09
R_{735}/R_{850}	3.30, **0.03**	5.82, **0.02**	2.67, 0.06
R_{740}/R_{850}	3.08, **0.04**	6.29, **0.02**[b]	3.15, **0.04**
R_{761}/R_{757}	7.57, **0.00**[a]	9.31, **0.01**[c]	2.13, 0.12
CI	3.46, **0.03**	1.13, 0.37	2.21, 0.10
D_{715}/D_{705}	3.36, **0.02**	0.97, 0.44	3.17, **0.04**
D_{max}/D_{745}	2.97, **0.04**	0.80, 0.54	1.28, 0.31
D_{705}/D_{722}	3.41, **0.03**	0.81, **0.53**	2.65, 0.06
R_{750}/R_{710}	3.93, **0.02**	0.65, 0.63	2.55, 0.07

[a] Significant differences at 30 mg, 100 mg, and 500 mg TNT kg^{-1} soil

[b] Significant differences at 250 mg and 500 mg TNT kg^{-1} soil

[c] Significant differences at 30 mg, 100 mg, 250 mg, and 500 mg TNT kg^{-1} soil

situations as an indicator of stress in plants; however, this appears to be a generalized stress response (i.e. plants exposed to drought, salinity, and flooding show decreases in PRI) that is tied to photosynthetic efficiency of the plant (Peñuelas and Filella 1998). Changes at R_{740}/R_{850}, as well as R_{735}/R_{850} may be attributed to the chlorophyll fluorescence peak at 740 nm as well as structural characteristics of leaf anatomy in the near infrared region which could be influenced by transformation and conjugation of TNT metabolites with lignin and incorporated into the cell wall (Burken 2003).Several other reflectance indices were able to detect some level of contamination in *M. cerifera*. The simple ratio R_{761}/R_{757} was the most successful index and to detected stress in all treatment levels. Reflectance at 760 nm has been demonstrated to be fluorescence *in-filling* of the 0_2 atmospheric absorption (Moya et al. 2004; Pérez-Priego et al. 2005; Zarco-Tejada et al. 2009). It is likely that the R_{761}/R_{757} was so useful because of this absorption, which allows for the relatively weak fluorescence signal to be differentiated from the more intense reflectance signals (Campbell et al. 2008). Reflectance remote sensing has the potential for detecting TNT induced stress in plants.

Many of the indices that were used to differentiate TNT induced stress from control plants have also been useful for detection of drought in other species, and, thus, the issue of spectral phenomena attributed to a specific plant-contaminant interaction still remains. However, the lack of change in certain spectral signatures and indices is also of note. There were no significant differences in indices linked specifically to water stress, which could help distinguish certain types of stress from drought. Changes in indices related to cell wall structure in the absence of chlorophyll changes (determined both from reflectance and extractions) could indicate the presence of explosives. Particularly, narrow band changes in the near-IR may be important for detecting xenobiotics, including TNT, and discriminating from other forms of mild to moderate natural stress. Further studies examining a combination of stressors (TNT and natural) are needed to determine if responses are in fact generalized or if any of these changes are separable from natural stress and to apply remote sensing at the canopy and landscape-level for the detection of explosives.

Acknowledgements This research was support by a grant to DRY from the United States Army Research Office. Spencer Bissett , Steven Brantley and Kati Rubis provided support with spectral reflectance collection and data processing. This research was also supported in part by an appointment for JCN to the Postgraduate Research Participation Program at the U.S. Army Corps of Engineers/Engineer Research and Development Center (ERDC) administered by the Oak Ridge Institute for Science and Education through an interagency agreement between the U.S. Department of Energy and ERDC.

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